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# Teratogenic Potential of Diazinon in Mice Embryos

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## SUMMARY

*A commonly used organophosphorous insecticide Diazinon was given to pregnant mice in doses of 5, 50, 100, 250 and 500 µg/g body weight on 7th day of gestation. Morphological study of embryos recovered on 15th day of gestation revealed that low doses (5 µg/g body weight) produced mild dwarfism and weight reduction. Higher doses (50-500 µg/g body weight) besides producing marked dwarfism and weight reduction, also resulted in retarded growth of specialized sense organs, limbs, snout and tail in a dose related manner. Doses of 100 µg/g body weight and above also produced toxic affects in mother. Survival from these effects was 100% in group C, 50% in group D and just 17% in group E. In conclusion Diazinon was found to be teratogenic to mice embryos producing growth retardation and underdevelopment of various organs in a dose related manner.*

## INTRODUCTION

Way Back in 1941, discovery by Gregg that German measles affecting a mother during pregnancy caused embryonic anomalies, stimulated research in the field of teratology. Since then many environmental factors including pesticides have been linked to teratogenesis[1].

Diazinon is an organophosphorous insecticide widely used as a soil and foliar insecticide and in household sprays, all over the world including Pakistan.

Our population is exposed to harmful effects of Diazinon by two main sources. One, by occupational exposure while using it on crops etc. and second by consuming uncooked and contaminated vegetables, fruits, grains and other foodstuffs. Toxic effects of organophosphates are known in humans and backed by various studies[2,3], but the embryotoxicity has mostly been studied in avian system[4].

The purpose of this study was to determine the embryotoxicity and teratogenicity of Diazinon, if any, using varying concentrations in mammalian placental system.

## MATERIAL AND METHODS

A total of 42 females and 14 males albino mice *Mus musculus* were used for present research. They were provided with commercially prepared food (chick feed no. 3) and water *ad libitum*.

A study of vaginal smears of female mice was carried out to detect the stage of estrus cycle. External appearance of female external genitalia also gave a clue to the state of estrus cycle. Mating was allowed in estrus period. Presence of vaginal plug or sperms in vagina were considered as signs of initiation of pregnancy.

The pregnant female mice were divided in various following groups, labelled and given oral doses of Diazinon accordingly.

**Control group**

- Group I: Plain control group.
- Group II: Vehicle control group.

Experimental animals were divided in five groups of six animals each and were given Diazinon in doses of 5, 50, 100, 250 and 500 µg/g body weight

(groups A to E).

These animals were given Diazinon in corn oil or just corn oil (vehicle control) on 7th day of gestation and were sacrificed on 15th day of gestation.

Uteri along with embryos were removed and fixed in Bouin's fixative. After 48 hours of fixation embryos were washed in 70% alcohol, were dissected out of uteri. They were cut and separated through umbilical cord, further washed in 70% alcohol till Bouin's fixative was completely alcohol off. Embryos obtained were then preserved in 80% alcohol.

Crown-rump length of embryos was measured using a millimeter scale. Weight was determined in milligrams on an electric balance. Detailed morphological study was then carried out under dissecting microscope using a magnification of 10x. Head, ear, eye, snout, limb structures trunk and tail were carefully seen and compared in different groups.

## RESULTS

### Controls

The 15 day old control embryos in both groups I and II were all well developed and normal with average crown-rump length of 13.27 mm and average weight of 0.381 grams.

### Experimental animals

#### Mothers

Diazinon, in addition to inducing growth retardation in embryos, also produced toxicity in mothers. Doses of 50 $\mu$g/g body weight and above (group C to E) produced signs ranging from mild to severe toxicity, in mothers, however survival of mothers was 100% in group C, 50% in group D and 17% in group E.

### Embryos

#### General appearance

In all the experimental groups, prominent feature was growth retardation indicated by overall reduction in size, weight and crown-rump length of embryos in a dose related manner (Fig. 1; Tables 1 and 2).

Head, trunk and tail regions were distinct from each other in all the treated embryos except for group E where the embryos consisted of just a small head bulge and a curved structure consisting of trunk and tail (Fig. 2).

**Table 1: Statistical analysis of crown-rump length of embryos using t-test.**

| *Groups* | *Mean* | *Means difference from control* | *Standard error* | *P value* |
|---|---|---|---|---|
| Control K | 13.27 | - | - | - |
| A | 12.78 | 0.49 | 0.153 | >0.05 |
| B | 11.15 | 2.12 | 0.133 | <0.05 |
| C | 9.42 | 3.85 | 0.119 | <0.01 |
| D | 9.42 | 10.03 | 1.10 | <0.01 |
| E | 0.769 | 12.50 | 0.31 | <0.001 |

**Table 2: Statistical analysis of weight of embryos using t-test.**

| *Groups* | *Mean* | *Means difference from control* | *Standard error* | *P value* |
|---|---|---|---|---|
| Control K | 0.381 | - | - | - |
| A | 0.320 | 0.061 | 0.036 | >0.05 |
| B | 0.195 | 0.186 | 0.033 | <0.05 |
| C | 0.135 | 0.246 | 0.046 | <0.05 |
| D | 0.010 | 0.371 | 0.027 | <0.001 |
| E | 0.0023 | 0.379 | 0.300 | <0.001 |

#### Head

In group A three brain vesicles were seen enclosed in developing skull. In groups B and C these brain vesicles were small but distinct from each other. Size of hind brain was reduced as the dose was increased. Suture lines of skull were prominent with wider fontanellae. In group E hind brain and skull was not visible at all.

#### Eye

In groups A and B an elliptical eye aperture with large rounded lens was seen covered by eyelids which were almost complete in group A, but had just started forming in group B. Eye aperture was elliptical in group C and rounded in group D with small lens and no identifiable eyelids in both. In group E there was just a small pinpoint elevation of underlying developing lens.

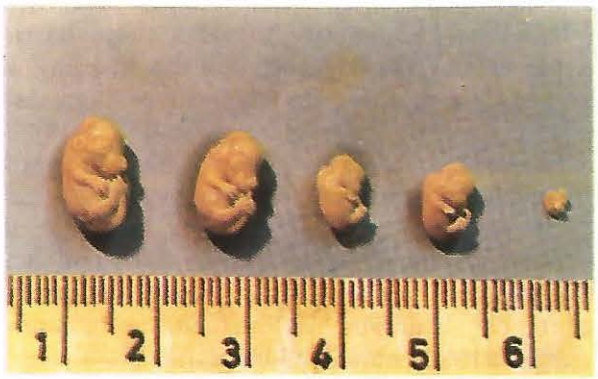


**Fig. 1: Photograph showing reduction in size and weight of mice embryos in experimental groups A, (left) to E, (right).**

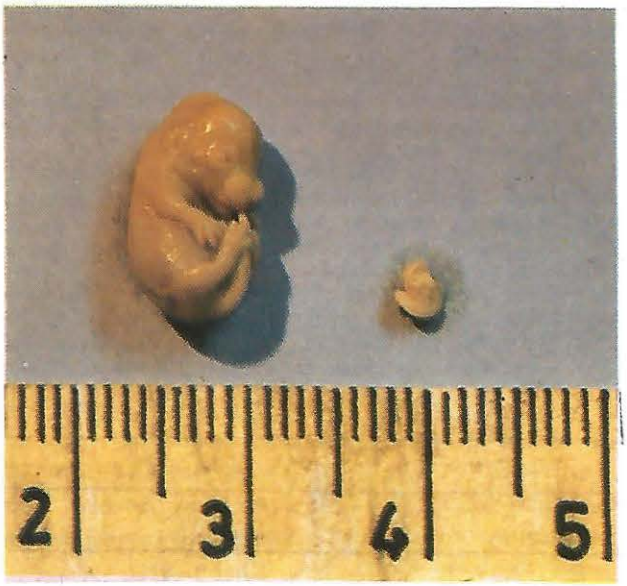


**Fig. 2: Photograph showing comparison of control (left) and group E (right) embryos.**

### Ear

In all the experimental groups external auditory meatus was seen but size of pinna was reduced in a dose related manner to the extent that in group E just a small pit was indicating the site of external auditory meatus with no signs of pinna formation.

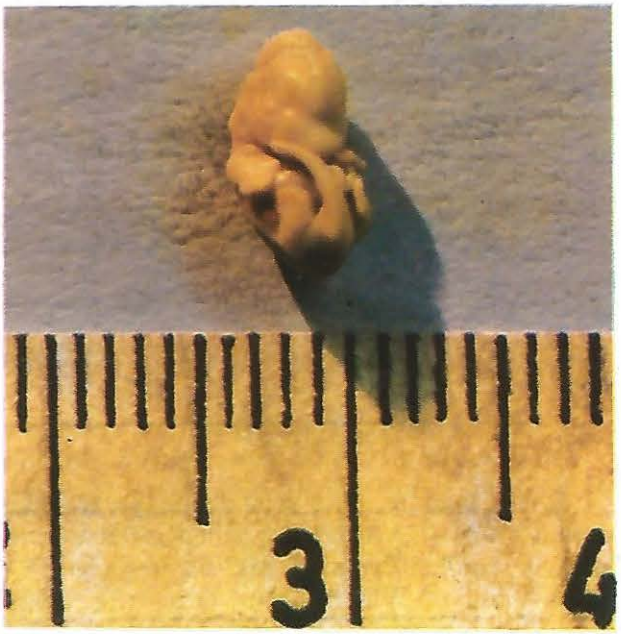


**Fig. 3: Photograph of ventral view of group D embryo given 250 μg/g body weight of diazinon showing prominent mid brain bulge, long tail and paddle like limb buds with no interdigital clefts.**

### Snout

In group A snout had taken its typical protruding shape with well developed lips, jaws and vibrissae. In groups B to D with increasing doses, nasal septum became thicker, lips wider, thick and flat, less prominent lower jaw and scanty vibrissae. In group E these structures had not formed at all.

### Trunk

In group A abdomen was protuberant because of underlying liver and thorax was flat as heart bulge was hidden by ribs. In group B because of thin skin and less developed ribs heart and liver bulges although small, were more clearly visible. However, in group E these bulges were very small.

### Limbs

In group A development of limbs was not affected. In group B knee and elbow bends had just become visible but were not formed in groups C and D. Interdigital clefts were not deep in group B while digits were not differentiated at all in groups C and D. In group D limbs were just in the form of straight strips ending in flat paddle like hand and feet. In

group E fore and hind limb buds had just appeared in the form of small flat flaps of tissue (Fig. 3).

**Tail**

In group A tail was similar to that in control groups. With increasing doses of Diazinon tail appeared more premature, thick and long reaching even beyond the facial area in group D. In group E tail was thick and not distinct from the trunk.

## DISCUSSION

It has become quite clear from the results of present study that Diazinon, when given in small doses is non-embryotoxic and non-teratogenic to mouse embryo. When higher doses are used it proves to be teratogenic.

While experimenting on mammalian embryos Robens studied the teratogenic potential of various insecticides. Diazinon was not found to be teratogenic to rabbits and hamsters in low doses[5].

Similarly Tauchi et al. gave Diazinon to rats and concluded that this compound did not produce any toxic effects in doses of 0.53-4.0 mg/kg[6].

The results of present study regarding group A are quite similar to those of Roben and Tauchi et al. In group A 5 $\mu$g/g body weight of Diazinon did not show signs of teratogenicity in mice embryos.

Dobbins tried a dose of 95.2 mg/kg of Diazinon in rats. Increased maternal mortality and reduced foetal development were noticed in the form of reduction in fetal and placental weight and development of hydronephrosis[7].

The results of Dobbins are again comparable with group C of our research animals, where 100 $\mu$g/g body weight of Diazinon showed growth retardation and arrest of differentiation. Maternal mortality was not increased however, reversible toxic affects appeared in mothers.

In none of the above mentioned studies doses more than 95.2 mg/kg have been tried. In this regard our observations as far as lower doses are concerned produced identical or not surprising results. Higher doses have provided additional information not only in fetus but also in the mothers.

Liver of the mother is capable of detoxifying the harmful agents entering the maternal body. In mammals placenta is a very strong barrier against many agents and affords further protection to offspring. Inspite of tis if the chemical concerned is in high enough quantity to cross these barriers, deleterious effects can be seen on the offspring. It is obvious that in those animals where there is no placental barrier, these insecticides will be able to produce even more severe abnormalities. This can be appreciated in results of studies on chick egg[8,9,10].

It can be concluded from this study that Diazinon inspite of its less cumulative and better biodegradable properties is hazardous to the mammalian embryos. It can still be potentially hazardous to the human population.

## REFERENCES


1. Moore KL. Causes of congenital malformation. In: The Developing Human. W.B. Saunders Company, Philadelphia. 1982; pp. 140-61.
2. Jamil H. Organophosphorous insecticide poisoning. JPMA 1989; 39: 27-30.
3. Abend Y, Goland S, Evron E, Sthoeger ZM, Geltner D. Acute renal failure complicating organophosphate intoxication. Ren Fail 1994; 16: 115-7.
4. Sternberg SS. The carcinogenesis, mutagenesis and teratogenesis of insecticide. Review of studies in animal and man. Pharm Ther 1979; 6: 147-66.
5. Robens JF. Teratogenic studies of carbaryl, Diazinon, Norea, Thiram in small laboratory animals. Toxicol Appl Pharmacol 1969; 15: 152-63.
6. Tauchi KN, Igarashi H, Kawanishi, Suzuki K. Teratological study of diazinon in the rat. Institute for Animal Reproduction, 1979; Japan. MRID 00131150.
7. Dobbins PK. Organic phosphate insecticides as teratogens in the rat. J Fla Med Assoc 1967; 54: 152-56.
8. Hayes Jr. WJ. Pesticide studies in man. Williams and Wilkins. Baltimore, London. 1982; pp. 56-82.
9. Fulton HM, Chambers. The toxic and teratogenic effects of selected organophosphorus compound on the embryos of three species of amphibians. Toxicol Letter 1985; 26: 175-80.
10. Seifert J, Casida JE. Progress in pesticide biochemistry (eds. Huston DH, Roberts TR), John Wiley and Sons, Ltd. vol. 1, 1981; pp. 219-41. .



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